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Administration of ACTH to restrained, pregnant sows alters their pigs' hypothalamic-pituitary-adrenal (HPA) axis[1,2]

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ABSTRACT: This study was designed to examine the physiology and behavior of pigs whose dams were snared and then injected with ACTH during gestation. Administration of ACTH to dams during pregnancy has been shown to replicate the effects of prenatal stress in other species. Control sows (n = 8) were given no treatment, whereas the treatment sows (ACTH, n = 8) were immobilized by snaring the snout and then administered an i.v. injection of ACTH (1 IU/kg BW) weekly from 6 to 12 wk of gestation. A pig was killed from each sow at 1, 30, and 60 d of age. The hypothalamus, pituitary gland, adrenal glands, and liver were immediately obtained to determine the amounts of corticotropin-releasing hormone (CRH), β-endorphin, and mRNA for pro-opiomelanocorticotropin (POMC), the ACTH receptor (ACTH-R), and insulin-like growth factor I (IGF-I). Pituitary corticotrope and somatotrope cell numbers and adrenal cortex-to-medulla area ratios (CORT:MED) were also determined. Pigs' behaviors were recorded at 6 and 8 wk of age. At 75 d of age, a blood sample was taken and a biopsy puncture was created on one pig from each litter, then pigs were stressed by mixing. Blood was sampled every other day for 10 d to determine plasma cortisol concentrations and differential leukocyte counts. Biopsy damage was evaluated for healing. At 1 d of age, control pigs tended to weigh more (P = .09), have a lower expression of ACTH-R mRNA (P = .01) and IGF-I mRNA (P = .01), and a lower CORT:MED (P = .04) than ACTH pigs. At 30 d of age, control pigs had a greater concentration of β-endorphin (P = .01) and tended to have a lower concentration of CRH (P = .09) and IGF-I mRNA (P = .10) than ACTH pigs. At 60 d of age, control pigs tended to have lighter pituitary glands (P = .08), a lower expression of POMC mRNA (P = .02), and a CORT:MED (P = .003) than ACTH pigs. At 8 wk of age, control pigs performed a higher frequency of belly nosing (P = .07) and oral vice behaviors (P = .01) than ACTH pigs. In response to mixing stress, control pigs had lesser concentrations of plasma cortisol (P = .03) and healed faster (P = .006) than ACTH pigs. Thus, exogenous ACTH and restraint during gestation alters the HPA axis of the sow's offspring, and during stressful situations later in life health, and therefore welfare, may be compromised.

Key Words: Adrenal Gland, Behavior, Corticotropin, Pigs, Stress

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Introduction

Stress results from reactions of the body to forces of a deleterious nature, infections, and various abnormal states that tend to remove it from homeostasis (Steadmans, 1995). In modern farming, many management practices are considered to be stressful to livestock species (Minton, 1994; von Borell, 1995). Confinement of swine is considered a potent chronic stress (Janssens et al., 1994). Chronic stresses to the pregnant dam may affect her developing fetus, a process termed prenatal stress.

Maternal glucocorticoids have been shown to cross the placenta in pigs (Klemcke, 1995), and in rats, they have been shown to cross the placenta and bind to the fetal hypothalamus (Zarrow et al., 1970). These maternal hormones have the potential to affect the maturation of the fetal hypothalamic-pituitary-adrenal (**HPA**) axis, which in turn may alter HPA function later in life.

Stressing pregnant rats causes many effects on the offspring, including alteration of emotional reactivity

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(Archer and Blackman, 1971), alteration of sexual behavior in males and females (Ward, 1972; Herrenkohl, 1979), and prolonged corticosterone secretion in response to restraint stress (Vallée et al. 1996). A limited amount of prenatal stress research has occurred in livestock. Lay et al. (1997a,b) reported that prenatally stressed calves had heavier body, pituitary gland, and heart weights and a greater increase in plasma cortisol and slower plasma cortisol clearance rates in response to stress compared to control calves.

Because other studies have reported that ACTH administration to pregnant dams replicates the effects of prenatal stress (Wilke et al., 1982; Lay et al., 1997a), the objective of this study was to determine differences in the physiology and behavior of pigs whose dams were restrained and administered ACTH from the 6th to 12th wk of gestation, compared with pigs from unmanipulated dams.

Materials and Methods

Animal Procedures

The procedures reported herein were approved by the Iowa State University Committee on Animal Care (2-8-3813-3-S). Nineteen Yorkshire × Landrace sows (184.5 ± 4.73 kg, 4.00 ± .42 parity) were bred to Duroc × Hampshire boars and were randomly assigned to either the control (n = 9) or the adrenocorticotropic hormone injected during restraint (ACTH; n = 10) treatments. Two ACTH-treated sows were not pregnant and one control sow died within the first 2 wk of the experiment; consequently, these sows could not be used in the study (remaining sows: control = 8, ACTH = 8). All sows were maintained in gestation stalls and subjected to normal management practices, with the exception that ACTH sows were administered ACTH (1-39, Corticotropin A; Sigma Chemical Co., St. Louis, MO) at a dose of 1 IU/kg of body weight at 1600 at 6, 7, 8, 9, 10, 11, and 12 wk after conception, whereas the control sows were left largely undisturbed during gestation. The time of administration was chosen because it has been shown that other species are susceptible to prenatal stress at this time (Schneider et al., 1992; Lay et al., 1997a), and the brain develops glucocorticoid receptors in different regions during different times throughout gestation (Rosenfeld et al., 1993). Because of this, administration of ACTH occurred over a large portion of pregnancy in order to possibly affect different developmental time points. The ACTH-treated sows were weighed weekly to determine the correct dose and then immobilized by snaring the snout so ACTH could be injected via the jugular vein. By administering the same dose of ACTH to each sow the resultant cortisol increase would be less variable because all sows would likely have similar cortisol profiles resulting from the ACTH injection. Conversely, cortisol release in response to a situational stress (restraint, unpredictable noise, etc.) is dependent on the animal's perception of that stress rather than a precise ACTH concentration. Although a snare was used to administer the ACTH injection, the high dose provided likely produced a maximal cortisol response, thereby reducing the effective stress of the snare restraint, although snaring could produce changes other than increased cortisol. However, this study was designed to determine whether ACTH administration during restraint to pregnant sows would affect the sow's subsequent offspring, and the precise mechanisms explaining what caused these effects were not examined.

To determine the sow's glucocorticoid response to the ACTH injection, two control sows and two ACTH sows were randomly selected and blood was collected via the jugular vein at 0, 30, 60, and 120 min after administration of treatments (ACTH and saline) during the 11th wk of gestation. These procedures were repeated with two different control and ACTH sows during the 12th wk of gestation to produce a total of four control sows and four ACTH sows sampled. Plasma was then frozen until it was assayed for cortisol (RIA; DPC, Los Angeles, CA).

Sows from both treatments farrowed in traditional farrowing stalls (Lage Products, Montezuma, IA) that were 1.5 × 2.3 m with a width of 61 cm for the sow and creep areas on both sides for piglets (45.72 cm × 2.3 m). All crates were equipped with plastic-coated, expanded metal flooring and a heat lamp, which was placed 20 cm away from the sow's shoulder. During farrowing, piglet birth intervals were recorded by time-lapse photography (1 frame/.4 s). Between 12 and 24 h after the completion of farrowing, piglets were weighed and sexed, and the anogenital distances were recorded. All other production data (e.g., stillborns, mummies, or low viability) were also recorded.

Within 24 h of birth (denoted 1 d of age) and at 30 and 60 d of age, one male pig of average weight for the entire litter was killed by exsanguination after sedation with 1.5 mL of xylazine (Fort Dodge Animal Health, Ford Dodge, KS). Males were chosen so that results would not be confounded by sex. All killings occurred at 0800 for any piglet born within the last 24 h. Because one ACTH-treated sow had a small litter with few males, no male from that litter was killed at the 30-d time period. Ten milliliters of blood was collected in heparinized tubes during exsanguination and then centrifuged at 2,600 rpm (approximately 770 × *g*) for 25 min at 4°C to collect plasma for quantification of cortisol concentrations by RIA. The hypothalamus, pituitary gland, and adrenal glands and two sections of the liver, heart, and testes were immediately obtained from the animal and weighed. The pituitary gland was only weighed at the 60-d period due to its small size at 1 and 30 d. The hypothalamus, half of the pituitary gland (severed mid-sagittally, yielding approximately equal amounts of posterior and anterior tissues in each section), the right adrenal gland, and both sections of the liver were placed in cryovials (Fisher Diagnostics, Pittsburgh, PA) and frozen in liquid nitrogen. These organs were collected to determine amounts of corticotropin-

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releasing hormone **CRH**, in hypothalamus), β-endorphin (in hypothalamus), CRH mRNA (in hypothalamus), pro-opiomelanocorticotropin mRNA (**POMC**, in pituitary gland), ACTH receptor mRNA (**ACTH-R**, in adrenal), and mRNA for the growth hormone receptor (**GH-R**), insulin-like growth factor I (IGF-I), and insulin-like growth factor II (IGF-II) in the liver. The other half of the pituitary gland and the left adrenal gland were fixed in 10% formalin (Fisher Chemical). The fixed pituitary tissues were sectioned and subsequently stained by immunocytochemistry (**ICC**) procedures to evaluate growth hormone (GH) and ACTH-specific cell populations. The fixed adrenal glands were cross-sectioned and stained with hematoxylin and eosin to determine the relative area of adrenal cortex and medulla for each animal.

The remaining piglets in each litter were weaned at 17.19 ± .36 d. After weaning, piglets were blocked by litter and four females and two males were placed in 1.22- × 1.17-m pen. Pigs that were not assigned to these pens were not used for the remainder of the study. Pens were all located in the same room, and each group of weaned pigs was located on one side of the room with treatments alternating in the eight pens. All pigs were observed to record behavioral data at 6 and 8 wk of age. Observations began at 1400 for a 15-min duration per pen to record duration of eating, frequency of drinking, frequency of play behavior, frequency of agonistic encounters (short encounter < than 5 s; long encounter > than 5 s), and frequency of abnormal behaviors, including manipulation of other pigs, rooting, belly-nosing, bar-biting, and tail-biting. Because actual ingestion of feed could not be measured, feeding activity was counted when the head of a pig was in the feeder. Likewise, drinking was counted every time the snout of a pig touched the automatic waterspout. Play was defined as exaggerated movements (e.g., head tosses, galloping), and agonistic encounters consisted of one individual attacking or fighting with another individual for either < or > 5 s. Manipulations were counted when a pig either nosed, bit, pushed, or suckled another pig's ear, head, leg, or ventral and dorsal surface. Rooting was counted when the snout contacted the floor and pushed forward. Belly nosing was counted when the snout contacted the belly of another pig and moved upward from the point of contact in a rooting type motion. Bar-biting or tail-biting was counted when the pig bit either the pen or feeder or another pig's tail, respectively. For analysis, orally fixated behaviors (belly nose, manipulation of other pigs, bar-biting, and tail-biting) were assessed individually and also combined as one class of behavior (oral vice). Pigs were observed one pen at a time by two trained observers, so throughout the observation period each pen was observed twice, 1 h apart. Piglets were also weighed once every 2 wk from weaning to 9 wk of age to record weight gain.

At 60 d of age, all pigs were moved into 2.65- × 1.77-m pens in the same arrangement as above. At 75 d of age, one female pig ranking second in dominance in each pen (n = 5 per pen) was used in a test to determine cortisol concentrations and immune function, in response to mixing stress. This age provided an opportune time to perform a stress test because at this research farm pigs are often moved, and hence mixed, at this age due to their increased size, and thus it provided a stressful situation that may actually take place within a production system. Dominance hierarchy was determined by removing feed for 1 d and then allowing access to only one feeder the following day. The most dominant pig, defined as the one having unchallenged access to the feeder or able to secure a position at the feeder for the longest duration (in every case this turned out to be a male), was marked and taken from the pen. The second most dominant animal (subdominant; in every case this turned out to be female) was then marked and immobilized by snaring the snout and a basal blood sample (10 mL) was immediately drawn via the jugular vein for quantification of cortisol concentrations and to determine differential leukocyte counts. After collecting the blood sample, a punch was used (6 mm, Miltex Instruments, Lake Success, NY) to create a biopsy puncture on the pig's right hindquarter. The biopsy puncture provides a functional test of neutrophil and macrophage function (Kiecolt-Glaser et al., 1995; Marucha et al., 1998). Because mixing of unfamiliar pigs is recognized as a strong social stress (Douglas et al., 1993; Moore et al., 1994; Stookey and Gonyou, 1994), these subdominant pigs were then randomly placed into a new pen within their own treatment. Blood samples were obtained every other day, at 0800, for a total of 10 d and the wound created by the biopsy was photographed for later scoring. Subdominant pigs were used because these animals are more severely stressed than dominant pigs in a recently mixed group (McGlone et al., 1993; Moore et al., 1994). All pens were kept in the same sanitary condition and animals were observed for signs of disease in order to reduce variation between pens. Only 14 of the 16 pens were used because two pens (1 control and 1 ACTH) had too few pigs, with four and three, respectively.

Radioimmunoassay

Cortisol concentrations were determined on duplicate samples using commercially available RIA double antibody kits (Diagnostics Products, Los Angeles, CA). Cross-reactivity of the cortisol antiserum was as follows: prednisolone, 89%; 11-deoxycortisol, 6.8%; cortisone, 3.9%; 6β-hydroxycortisol, 3.6%; and corticosterone, prednisone, and dexamethasone, < 1.1% (analyses by DPC). The kits were used according to the manufacturer's specifications. Precision and accuracy of this assay were evaluated in triplicate using a swine plasma pool containing approximately 139.5 ng/mL of cortisol, resulting in an intraassay CV of 5.8% and an interassay CV of 7.9%.

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Levels of CRH in hypothalamic extracts were quantified using a heterologous double-antibody RIA outlined by Peninsula Laboratories (Belmont, CA). Samples of extract were quantified in duplicate 100-μL aliquots. Samples or standards of porcine CRH and 100 μL of rabbit anti-CRF (human, rat) antiserum were added to each tube and allowed to incubate at 4°C overnight. Radiolabeled [^{125}I]hCRF-Tyr at approximately 15,000 cpm/100 μL was added to each tube and again incubated overnight at 4°C. Then, 100 μL of goat anti-rabbit IgG (GARGG-500) and normal rabbit serum (NRS-500) was added to each tube, and samples were incubated for 2 h at room temperature. A quantity (500 μL) of RIA buffer was added to each tube before centrifugation at 1,700 × *g* for 20 min at 4°C. The CRH concentrations (pg/mg tissue) were calculated by comparison to a standard curve generated by serial dilutions (128 pg/100 μL to 0.125 pg/100 μL) of porcine CRH. Details of these procedures have been published (Bishop et al., 1999).

Concentration of β-endorphin in hypothalamic extracts were quantified using a heterologous double-antibody RIA similar to that of CRH. The sequence of steps was the same as for the CRH assay except that rabbit-anti-β-endorphin (rat) serum was used as the first antibody, the radiolabeled ligand was porcine β-endorphin, and 750 μL of 6% polyethylene glycol was added in lieu of 500 μL of assay buffer. Details of these procedures have been published (Leshin and Malven, 1984).

Quantification of mRNA

Total RNA was extracted from liver, pituitary, and adrenal glands (Tri-Reagent, Molecular Research Center, Cincinnati, OH) and transferred to a nylon membrane with a slot-blot apparatus (Bio-Dot SF, Bio-Rad Laboratories, Hercules, CA). The slot-blot analyses do not produce data on relative abundances of major vs minor transcripts; thus, any significant treatment effect in the present study reveals a change in overall transcription of the gene product. Hybridization and detection were carried out with a commercially available kit according to the manufacturer's instructions (BrightStar System, Ambion, Austin, TX). Hybridization signal intensities were quantified by densitometry, and target mRNA values were expressed relative to 28s rRNA for each sample. The benefit of using 28s rRNA as a normalization control is that any possible cross-hybridization to rRNA will be canceled and not contribute to producing an erroneous treatment effect. In addition, although background issues are a problem with rare transcripts, the target mRNA in the present study are not of low abundance.

Polymerase chain reaction (PCR) was used to amplify POMC and ACTH receptor cDNA (RNA-PCR kit, Perkin-Elmer, Foster City, CA). The forward and reverse oligonucleotide primers for PCR amplification were 5′ GTG GGA GAT GCC GAG ATT GT 3′ and 5′ CTC CTC CTC CTC GCG CTT CT 3′ for POMC (343 bp) and 5′ TCT GTG ATT GCC GCT GAC CG 3′ and 5′ TTT TTG AAT GCG ACC CTG AG 3′ for the ACTH receptor (501 bp; GenBank accession no. AF064077). The PCR products were cloned into a T-cloning vector (PCR-II, Invitrogen, San Diego, CA). The identities of the cDNA clones were determined by dideoxy termination sequencing. The POMC sequence is identical to that previously reported for pigs (GenBank accession no. S73519). The porcine ACTH receptor sequence is 85% homologous to the human ACTH receptor cDNA sequence (GenBank accession no. X65633). Biotinylated riboprobes were synthesized from these clones for chemiluminescence-based detection using a commercially available kit (BrightStar System). Details of these procedures have been published (Daniel et al., 1999).

Immunocytochemistry

The methods of Childs and Unabia (1982) were followed when performing ICC work on the pituitary gland tissues. The tissues were embedded in paraffin within 2 wk of fixation. Each pituitary gland was sectioned onto two slides, each slide containing six sections, and was then deparaffinized, labeled with antibody to either ACTH (1:1,000 dilution; Sigma) or GH (1:2,000 dilution; Biogenesis, Poole, U.K.), and stained using the avidin-biotin complex (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA) and peroxidase (DAB substrate kit for peroxidase; Vector Laboratories). The antibody to ACTH shows positive binding to ACTH(18–39)-BSA, ACTH(1–39), and ACTH(18–39) and does not bind to BSA, ACTH(1–17), ACTH(1–10), FSH, TSH, GH, LH, or prolactin (analysis provided by Sigma Chemical). Using a projection microscope (XM150 Kramer Scientific, Elmsford, NY) at 400 × magnification, images of sectioned and stained pituitary glands were projected upon a wall and cells containing ACTH or GH were quantified. Three of the six sections were randomly chosen and three fields of view were counted from each of the three sections, for a total of nine fields. The edge of the tissue section was avoided when counting cells because these cells stained darker and thus counting would have been less accurate. Although observers were blind to treatments, two people counted the ACTH and GH cells in each field. Their values were averaged to control for variation between counters. The CV for counts were 6.16% for ACTH cells and 3.19% for GH cells.

Hematoxylin and Eosin Y Staining

Adrenal glands were cross-sectioned, yielding approximately two equal halves, and then placed upright on the cleaved surface and embedded in paraffin within 2 wk of fixation. Four adrenal sections were placed on a slide, deparaffinized, and stained using hematoxylin and eosin Y (1%) to allow for visualization of both the adrenal cortex and medulla. Using a projection microscope (XM150, Kramer Scientific) at 25× power, two of the four sections were randomly chosen and images of

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sectioned and stained adrenal glands were projected upon a wall, where the adrenal capsule and the line of demarcation between the cortex and medulla could be traced along with any open space where a blood vessel resided. The CV for the area of the two sections within the same gland were 6.36% for the adrenal cortex and 5.21% for the adrenal medulla. The tracings were then used to quantify the area of the entire gland, the cortex, and medulla using an image analysis system (Bioquant, Nashville, TN). The cortex:medulla ratio (**COR:MED**) was then compared between treatments and periods to determine differences.

Immunological Procedures

A portion of the blood collected from subdominant pigs during the mixing stress test was used to produce a blood smear on a slide and then stained using a Gram Stain Set (Fisher Diagnostics) to examine leukocyte differentials. Neutrophils, lymphocytes, macrophages, basophils, and eosinophils were counted on a microscope (Henry Louis, Iowa City, IA) using a 100× magnification oil immersion lens. A total of 100 leukocytes were counted from each slide, producing a percentage of the different cell types. Leukocyte percentages and the neutrophil:lymphocyte ratio (**N:L**) were then compared between treatments and time periods. Neutrophil:lymphocyte ratios have been reported as a reliable indicator of stress (Stull et al., 1999).

Biopsy punctures were evaluated on a scoring basis by indirect observation with the use of picture slides taken of the wound. Wounds were evaluated using two indices, inflammation and healing ability. For inflammation, a score of 1 denoted no inflammation, 2 = slight pink ring around the wound, 3 = deep red ring around the wound, 4 = deep red ring around the wound and pus. Healing ability score was dependent on how much of the puncture created by the punch biopsy was filled in/healed; a score of 1 = the wound is at the level of surrounding tissue, 2 = the wound is above the level of surrounding tissue, 3 = the wound is below the level of surrounding tissue, 4 = no healing. The biopsy punctures were evaluated by three observers who were blind to treatments.

Statistical Analysis

All means are presented as means ± standard error. All data were analyzed with sow as the experimental unit and tests were performed to assess the feasibility of normality using the univariate procedure of SAS. Normal data were analyzed using the General Linear Models procedure of SAS and non-normal data were analyzed using the Wilcoxon-Mann-Whitney ranked sum test of SAS (1985). Production data (litter size; number born alive, number crushed, and number stillborn; weaning age; gestation length; and birth intervals), behavioral data, and scores for biopsy punctures were analyzed using the Wilcoxon-Mann-Whitney test. Birth weights were analyzed using the General Linear Models procedure and accounting for repeated measures, with treatment, sex, treatment × sex, and treatment nested within sow included in the model. Anogenital distances were analyzed using the General Linear Models procedure and accounting for repeated measures, with birth weight, treatment, and treatment nested within sow included in the model. Weights obtained every 2 wk and average daily gain were analyzed using the General Linear Models procedure and accounting for repeated measures, with treatment, sex, time, treatment × time, and treatment nested within sow included in the model. Organ weights were analyzed using the General Linear Models procedure, with treatment, time, and treatment × time included in the model.

All mRNA for GH receptor, IGF-I, IGF-II, POMC, and ACTH receptor data as well as CRH, β-endorphin, and cortisol data were analyzed using the General Linear Models procedure and accounting for repeated measures, with treatment, period, treatment × period, and treatment nested within sow included in the model. The adrenal COR:MED ratio data were analyzed using the General Linear Models procedure and accounting for repeated measures, with treatment, period, treatment × period, treatment nested within sow, period nested within adrenal section, and period nested within treatment × section included in the model. Leukocyte data were analyzed using the General Linear Models procedure and accounting for repeated measures, with treatment, day, treatment × day, and treatment nested within sow included in the model. Neutrophil:lymphocyte ratio data were analyzed using the General Linear Models procedure and accounting for repeated measures, with treatment, day, treatment × day, and treatment nested within sow included in the model. Finally, cell number data that were visualized by ICC were analyzed using the General Linear Models procedure and accounting for repeated measures, with treatment, period, treatment × period, and treatment nested within sow included in the model.

Results

Sow Cortisol Concentrations

During the 11th and 12th wk of gestation, the four ACTH sows whose blood was collected via the jugular vein at 0, 30, 60 and 120 min after administration of ACTH had greater concentrations of plasma cortisol than the four control sows that were administered saline (ACTH = 159.84 ± 16.06 ng/mL, control = 57.54 ± 2.70 ng/mL; P = .0002). Furthermore, there was a treatment × time interaction such that time 0 was not significantly different between treatments (P = .30), but at times 30, 60, and 120 min ACTH sows had greater concentrations of cortisol than control sows (P = .0001; Figure 1).

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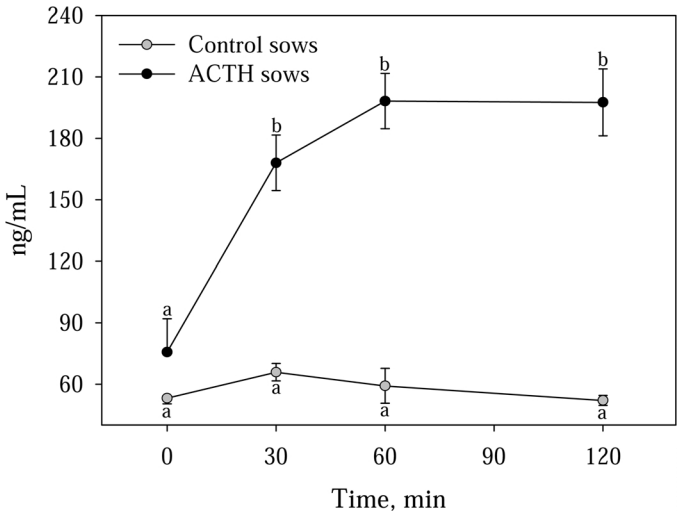

Figure 1. Sow plasma cortisol concentrations after administration of adrenocorticotropic hormone (ACTH) to ACTH sows (n = 4) and saline to control sows (n = 4) during the 11th and 12th wk of gestation. [a,b]Means ± SEM with different superscripts differ (P = .0001).

Production Data

There was no difference in litter size (11.25 ± .81 pigs per litter), number of piglets born alive (10.31 ± .73 pigs per litter), stillbirths (.94 ± .38 pigs per litter), or crushing (.38 ± .15 pigs per litter) between control and ACTH sows (P > .5). In addition, gestation length did not differ between control and ACTH sows (115.38 ± .34 d; P = .23). Piglet birth interval also was not different between control and ACTH sows (22.33 ± 3.76 min; P = .69).

Weights and Anogenital Distances

Control pigs tended to have greater (P = .09) birth weights than ACTH pigs (control = 1.67 ± .04 kg, ACTH = 1.41 ± .04 kg). At birth, anogenital distance did not differ between female control or ACTH pigs (10.35 ± .30 mm; P = .20) or male control and ACTH pigs (127.71 ± 2.01 mm; P = .90). Weights at 3, 5, 7, and 9 wk of age did not differ (P = .72) between control and ACTH pigs (3 wk = 5.82 ± .15 kg, 5 wk = 8.22 ± .22 kg, 7 wk = 16.42 ± .37 kg, 9 wk = 23.70 ± .46 kg). Likewise, average daily gain was not different (P = .98) between control and ACTH sows (.26 ± .01 kg).

Behavioral Observations

At 6 and 8 wk of age, no differences were found between control and ACTH pigs (P > .12) for frequency of tail biting, manipulation of other pigs, rooting, bar biting, short agonistic encounters, long agonistic encounters, drinking, or play (Tables 1 and 2). In addition, time spent feeding was not different between treatments (P > .37; Tables 1 and 2). Although there was no difference in the frequency of belly-nosing at 6 wk of age between treatments, at 8 wk of age frequency of belly-nosing tended to be greater (P < .07) for control pigs than for ACTH pigs (Table 2). Furthermore, there were no differences in the frequency of oral vices (P = .16) between control and ACTH pigs at 6 wk of age (Table 1), but at 8 wk of age the control pigs performed a higher frequency of the oral vice behaviors than the ACTH pigs (P = .01; Table 2).

Organ Weights

Because body weight differed between treatments, organ weight data were analyzed as organ weight:body weight ratios. Adrenal glands, testes, and hearts obtained from killed pigs did not differ in weight (P > .40) between the control and ACTH pigs at 1, 30, or 60 d of age. However, pituitary glands tended to be lighter in control pigs than in ACTH pigs at 60 d of age (control = 5.75 ± .48 mg/kg, ACTH = 6.82 ± .29 mg/kg; P = .08).

Anterior Pituitary Gland Immunocytochemistry and Adrenal Gland Morphology

There were no differences in the number of immunopositive ACTH (P = .60) or immunopositive GH (P = .73) cells in pituitary sections between control and ACTH pigs at 1, 30, and 60 d of age. The number of immunopositive ACTH and GH cells did decrease by period, however (P = .0001; Figure 2), suggesting that the number of corticotropes and somatotropes in the anterior pituitary decrease during the first 2 mo of life. There was also a greater number of immunopositive GH cells than immunopositive ACTH cells during the 1-, 30-, and 60-d periods (P = .0001; Figure 2).

The areas of the adrenal cortex for control pigs at 1, 30, and, 60 d of age were 282.25 ± 53.50, 514.11 ± 37.36, and 988.56 ± 71.96 mm^3, respectively, whereas the areas of the adrenal cortex for ACTH pigs were 300.69 ± 29.01, 489.76 ± 34.26, and 1,151.24 ± 65.59 mm^3 at the same time points. The areas of the adrenal medulla for control pigs at 1, 30, and 60 d of age were 92.52 ± 25.15, 104.61 ± 5.19, and 199.80 ± 11.91 mm^3, respectively, whereas the areas of the adrenal medulla for the ACTH pigs were 86.21 ± 7.68, 102.62 ± 12.35, and 186.55 ± 11.76 mm^3 at the same time points. The COR:MED was less in control pigs than in ACTH pigs at 1 d of age (control = 1.70 ± .74, ACTH = 3.54 ± .48; P = .04). However, at 30 d of age there was no treatment difference (control = 5.25 ± .35, ACTH = 5.09 ± .35; P = .75), but again at 60 d of age the COR:MED was less in control pigs than in ACTH pigs (control = 5.13 ± .30, ACTH = 6.45 ± .30; P = .003; Figure 3). Both control and ACTH pigs had similar increases of CORT:MED over the 60-d period, but ACTH pigs had a larger CORT:MED at birth than control pigs. Between 1 and 30 d of age the COR:MED of control pigs increased substantially (P = .0001), but there was no increase between 30 and 60 d of age (P = .80). Conversely, the COR:MED of ACTH

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Table 1. Behavioral data collected during direct observation of 6-wk-old control pigs and pigs whose dams were restrained and administered adrenocorticotropic hormone (ACTH) during gestation (ACTH pigs) for a 15-min duration beginning at 1400; for specific definitions of behaviors see text

Behavior	Control pigs	ACTH pigs	*P*-value
Belly-nosing, frequency	8.37 ± 4.67	4.13 ± 2.84	.72
Manipulating others, frequency	65.00 ± 15.33	61.50 ± 8.90	.79
Bar-biting, frequency	4.63 ± .84	27.00 ± 15.56	.49
Tail-biting, frequency	1.63 ± .63	2.00 ± .98	1.00
Oral vice, frequency	79.63 ± 17.71	94.63 ± 9.87	.16
Rooting, frequency	3.13 ± 1.27	2.63 ± 1.43	.70
Short agonistic behavior, frequency	4.25 ± 2.36	1.13 ± .61	1.00
Long agonistic behavior, frequency	5.88 ± 5.31	1.63 ± .96	.77
Play, frequency	4.25 ± 2.36	1.13 ± .61	.23
Drinking, frequency	12.75 ± 2.52	12.13 ± 2.43	.96
Feeding, duration, min	8.27 ± 2.67	10.66 ± 2.21	.56

pigs increased between 1 and 30 d of age ($P = .01$) and 30 and 60 d of age ($P = .005$), displaying a differential adrenal gland growth rate between control and ACTH pigs.

Hormone and mRNA Data

At 30 d of age, control pigs had greater concentrations of hypothalamic β-endorphin than ACTH pigs (control = 17.18 ± 2.51 ng/g tissue, ACTH = 9.57 ± 1.50 ng/g tissue; $P = .01$; Figure 4) and lower concentrations of hypothalamic CRH than ACTH pigs (control = .77 ± .25 ng/g tissue, ACTH = 1.26 ± .11 ng/g tissue; $P = .09$; Figure 4). Corticotropin-releasing hormone and β-endorphin were not measured at the 1- and 60-d time periods.

At 1, 30, and 60 d of age, there was no difference in the concentration of mRNA for GH-R (1.31 ± .05 arbitrary units), IGF-II (1.38 ± .06 arbitrary units), or CRH (.79 ± .06 arbitrary units; $P > .27$). Overall, for 1, 30, and 60 d of age, IGF-I mRNA tended to be lower in control pigs than in ACTH pigs (control = 1.00 ± .03 arbitrary units, ACTH = 1.11 ± .04 arbitrary units; $P = .09$). Specifically, IGF-I mRNA was lower for control pigs than for ACTH pigs at 1 d of age (control = .95 ± .06 arbitrary units, ACTH = 1.16 ± .06 arbitrary units; $P = .01$) and tended to be less for control pigs than for ACTH pigs at 30 d of age (control = 1.03 ± .06 arbitrary units, ACTH = 1.17 ± .06 arbitrary units; $P = .10$), and the treatment difference did not occur at 60 d of age ($P = .11$; Figure 5). There were no treatment differences for concentrations of POMC mRNA at 1 and 30 d of age, but by 60 d of age control pigs had lower concentrations of POMC mRNA than ACTH pigs (control = 2.27 ± .22 arbitrary units, ACTH = 3.06 ± .22 arbitrary units; $P = .02$; Figure 6). Finally, ACTH-R mRNA was lower in control pigs than in ACTH pigs at 1 d of age (control = .82 ± .12 arbitrary units, ACTH = 1.34 ± .13 arbitrary units; $P = .01$; Figure 7).

Plasma Cortisol Concentrations

Plasma cortisol concentrations derived from blood at exsanguination did not differ between control and ACTH pigs at 1, 30, or 60 d of age (control = 111.30 ± 6.38 ng/mL, ACTH = 121.05 ± 12.06 ng/mL; $P = .19$).

Table 2. Behavioral data collected during direct observation of 8-wk-old control pigs and pigs whose dams were restrained and administered adrenocorticotropic hormone (ACTH) during gestation (ACTH pigs) for a 15-min duration beginning at 1400; for specific definitions of behaviors see text

Behavior	Control pigs	ACTH pigs	*P*-value
Belly nosing, frequency	127.00 ± 81.60	2.13 ± 1.86	.07
Manipulating others, frequency	30.75 ± 11.15	10.38 ± 3.49	.27
Bar-biting, frequency	6.75 ± 2.27	2.88 ± .83	.27
Tail-biting, frequency	2.25 ± 1.86	.88 ± .52	.85
Oral vice, frequency	166.75 ± 74.66	16.25 ± 4.23	.01
Rooting, frequency	6.50 ± 3.53	5.63 ± 5.63	.12
Short agonistic behavior, frequency	3.38 ± 1.68	1.63 ± .68	.48
Long agonistic behavior, frequency	.00 ± .00	.25 ± .25	.38
Play, frequency	5.38 ± 5.23	.13 ± .13	.54
Drinking, frequency	2.38 ± 0.56	3.88 ± .85	.12
Feeding, duration, min	11.69 ± 2.11	8.94 ± 2.82	.37

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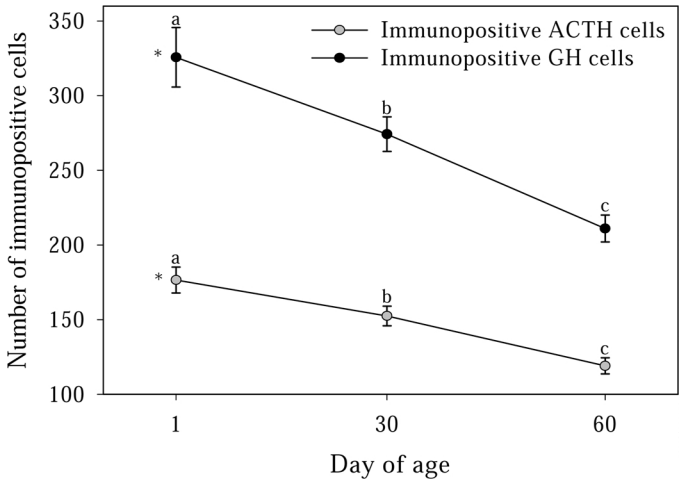

Figure 2. Number of immunopositive adrenocorticotropic (ACTH) and growth (GH) hormone cells from anterior pituitary glands at 1 (n = 16), 30 (n = 15), and 60 (n = 16) d of age. [a,b,c]Means ± SEM with different superscripts within the same measure differ ($P = .0001$). *Means ± SEM in different measures differ at each day of age ($P = .0001$).

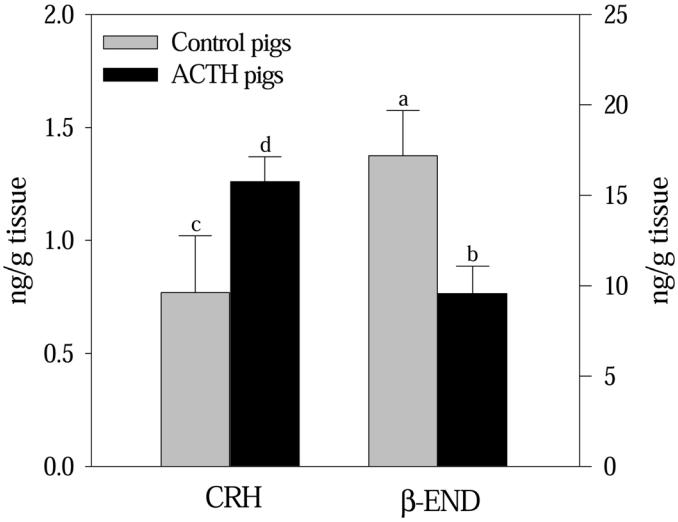

Figure 4. Corticotropin-releasing hormone (CRH) and β-endorphin (β-END) in the hypothalamus at 30 d of age from control pigs (n = 8) and pigs whose dams were restrained and administered adrenocorticotropic hormone (ACTH) during gestation (ACTH pigs; n = 8). [a,b-]Means ± SEM with different superscripts differ ($P = .01$); [c,d]means ± SEM with different superscripts tend to differ ($P = .09$).

Plasma cortisol concentrations were greatest at 1 d of age (145.38 ± 10.30 ng/mL) and had declined by 30 and 60 d of age (89.85 ± 6.98 ng/mL and 110.73 ± 11.65 ng/mL, respectively; $P < .02$).

Stress Test

Plasma Cortisol Concentrations. Control pigs had lesser concentrations of plasma cortisol than ACTH pigs during the mixing stress (control = 59.61 ± 3.83 ng/mL, ACTH = 70.58 ± 3.79 ng/mL; $P = .03$). Although there was no treatment × day interaction, it seems that plasma cortisol concentrations in ACTH pigs remained

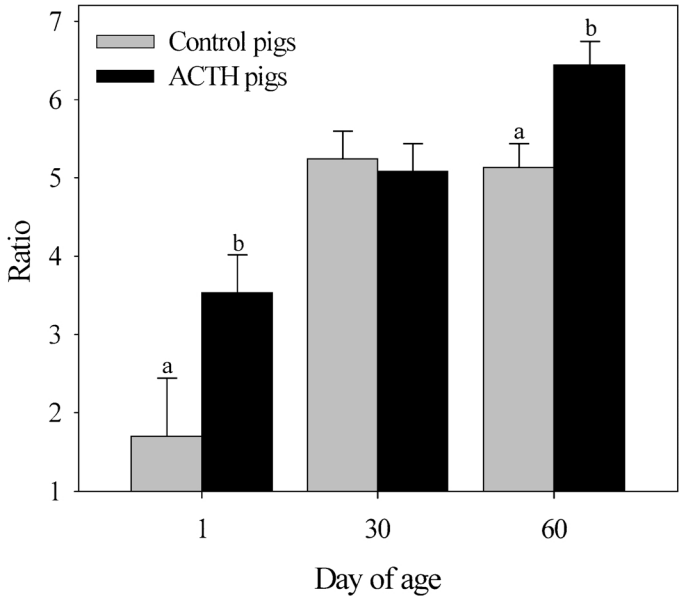

Figure 3. Adrenal cortex:medulla ratio at 1, 30, and 60 d of age (n = 8 per treatment at each time period). [a,b]Means ± SEM with different superscripts differ ($P < .04$). Means ± SEM differ with day of age ($P = .0001$).

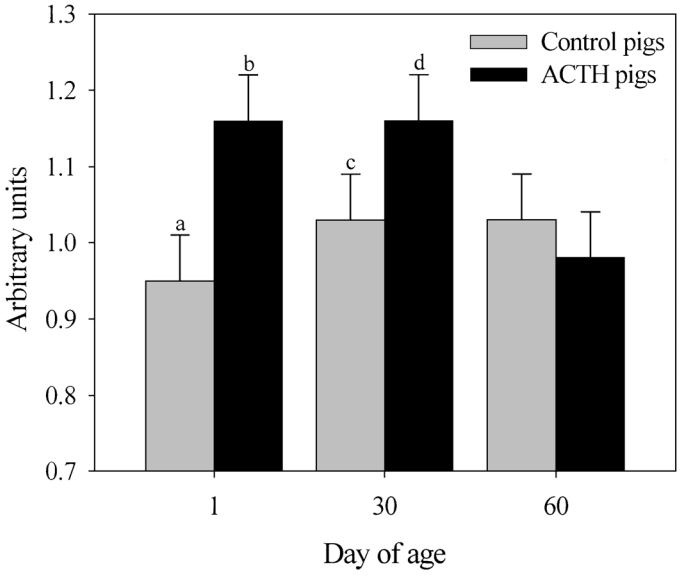

Figure 5. Insulin-like growth factor I mRNA in the liver at 1, 30, and 60 d of age (n = 8 per treatment at each time period). [a,b]Means ± SEM with different superscripts differ ($P < .01$); [c,d]means ± SEM with different superscripts tend to differ ($P < .10$). Means ± SEM do not differ with day of age ($P = .31$).

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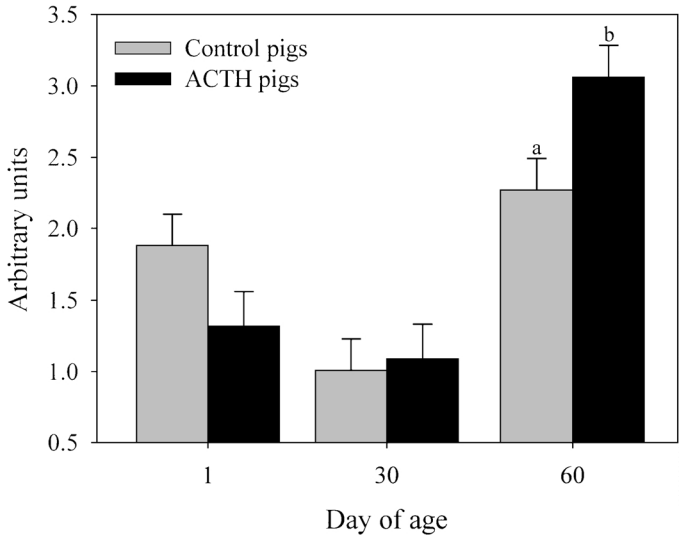

Figure 6. Pro-opiomelanocortin mRNA in the pituitary gland at 1, 30, and 60 d of age (n = 8 per treatment at each time period). [a,b]Means ± SEM with different superscripts differ ($P < .02$). Means ± SEM differ with day of age ($P = .0001$).

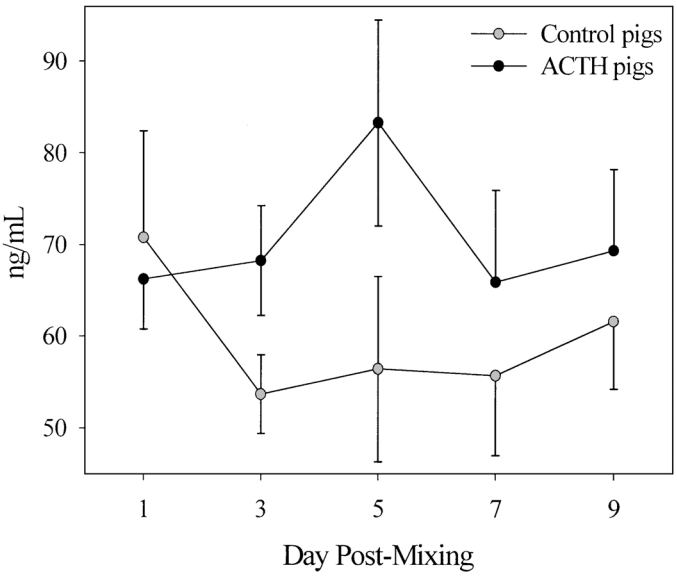

Figure 8. Plasma cortisol concentrations in response to mixing stress test for control pigs (n = 7) and pigs whose dams were restrained and administered adrenocorticotropic hormone (ACTH) during gestation (ACTH pigs, n = 7; $P = .03$). On d 1, after dominance hierarchy was determined in each pen, the subdominant pig was immobilized by snaring the snout and a basal blood sample was drawn; at this time a punch biopsy was performed. These animals were then placed in a new pen with unfamiliar animals.

higher than in control pigs until d 5 of the mixing stress (Figure 8).

Evaluation of Biopsy Puncture. Observation of punch wounds showed that control pigs healed more slowly than ACTH pigs (control = 2.47 ± .06 d, ACTH = 2.77 ± .09 d; $P = .006$). Specifically, d 3, 5, and 7 did not differ between treatment groups ($P > .56$), but control pigs had lower healing and inflammation evaluation scores than ACTH pigs at d 9 (control = 1.70 ± .10, ACTH = 2.78 ± .10; $P = .0001$) and d 11 (control = 1.83 ± .15, ACTH = 2.94 ± .15; $P = .0001$; Figure 9).

Leukocyte Differentials. There was no difference in the percentage of neutrophils, lymphocytes, monocytes, basophils, or eosinophils between control and ACTH pigs on d 1, 3, 5, 7, or 9 of the mixing stress ($P > .18$; Table 3). The N:L did not differ between control and ACTH pigs on any day of the mixing stress ($P = .39$).

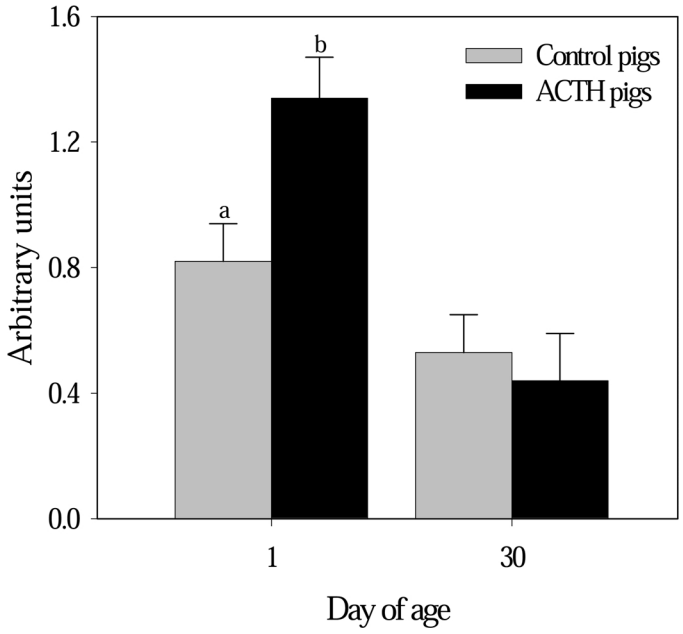

Figure 7. Adrenocorticotropic hormone receptor mRNA in the adrenal gland at 1, 30, and 60 d of age (n = 8 per treatment at each time period). [a,b]Means ± SEM with different superscripts differ ($P < .01$). Means ± SEM differ with day of age ($P = .001$).

Discussion

The results of this study indicate that restraint coupled with administration of exogenous ACTH during gestation alters the HPA axis of the sow's subsequent offspring. Similar to previous research, this study indicates that administration of ACTH to pregnant dams replicates the effects of various prenatal stressors, including restraint and heat (Wilke et al., 1982), unpredictable noise (Schneider et al., 1992), and transportation (Lay et al., 1997a,b). The finding that ACTH administration is similar to prenatal stress suggests that although stressors cause the synthesis and release of many hormones in the pregnant female, the predominant causal agent of prenatal stress may be activation of the HPA axis. In the current study, however, immobi-

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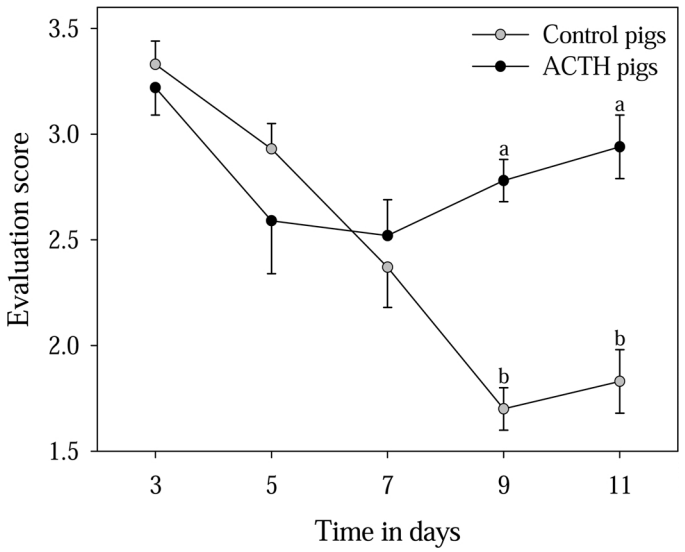

Figure 9. Indirect evaluation score of biopsy puncture for inflammation and healing during mixing stress test for subdominant control pigs (n = 7) and pigs whose dams were restrained and administered ACTH during gestation (ACTH pigs; n = 7). Biopsy puncture was produced on d 1 and evaluated initially on d 3. [a,b]Means ± SEM with different superscripts differ ($P < .0001$).

lization by snaring the snout so that ACTH could be injected via the jugular vein likely resulted in other hormones being released, which may possibly have caused prenatal stress. However, this study served as a preliminary experiment to determine whether prenatal stress effects exist in swine, and therefore the exact mechanism of the effect was not a primary consideration.

In this study, it was shown that in swine restraint coupled with ACTH injections from the 6th to 12th wk of gestation resulted in a hyperactive HPA axis compared to control pigs. At the level of the hypothalamus, ACTH pigs had greater concentrations of CRH than control pigs at birth. At the level of the pituitary gland, ACTH pigs had an increased pituitary gland weight and greater levels of POMC mRNA than control pigs at 60 d of age. At the level of the adrenal glands, ACTH pigs had greater levels of ACTH-R mRNA at 1 d of age and a greater adrenal COR:MED at 1 and 60 d of age than control pigs. Furthermore, hyperactivity of the HPA axis was demonstrated by a greater concentration of plasma cortisol in response to the mixing stress at 75 d of age in ACTH pigs.

In this study, restraint and ACTH administration to sows caused a threefold increase in plasma cortisol concentrations at the 30-, 60-, and 120-min time periods at the 11th and 12th wk of gestation. Plasma cortisol concentrations were still maximal at the last sampling period, which indicates that administration of 1 IU/kg BW (equivalent to .08 μg/kg BW and approximately 200 to 250 IU per sow) of ACTH effectively raised plasma cortisol concentrations for a minimum of 2 h.

In the hypothalamus, the concentration of CRH was greater at 30 d of age, whereas β-endorphin was decreased in ACTH pigs compared to control pigs. Hypothalamic β-endorphin is cleaved from POMC and is believed to function as a neuromodulator and neurotransmitter within the central nervous system (Norris, 1997). In addition, hypothalamic endogenous opioid peptides, such as β-endorphin, inhibit the release of CRH in response to stress by a direct action on CRH terminals in the paraventricular nucleus of the hypothalamus (Plotsky, 1986; Chrousos, 1992). Thus, the decreased concentration of hypothalamic CRH in control pigs may have resulted from the increased concentration of hypothalamic β-endorphin.

At the level of the pituitary gland, control pigs tended to have lighter pituitary glands than ACTH pigs at 60 d of age, which is in agreement with other studies in cattle (Lay et al., 1997b). Furthermore, ACTH pigs had greater concentrations of POMC mRNA, likely due to the greater concentrations of CRH. Corticotropin-releasing hormone has been shown to increase the rate of synthesis of POMC in vitro. Chronic administration of CRH to intact rats results in increased levels of POMC mRNA in the anterior pituitary gland (Liotta and Krieger, 1990).

Although there was no treatment differences in the number of immunopositive ACTH or GH cells in anterior pituitary gland sections at 1, 30, or 60 d of age, there were more immunopositive GH than ACTH cells. This is in accord with the greater concentration of somatotropes (~ 50%) than of corticotropes (~ 10 to 15%) in

Table 3. Mean ± SEM percentages of lymphocytes, neutrophils, monocytes, eosinophils, and basophils from subdominant control pigs and pigs whose dams were restrained and administered adrenocorticotropic hormone (ACTH) during gestation (ACTH pigs) during mixing stress

Leukocyte type	Control pigs	ACTH pigs	*P*-value
	%		
Lymphocyte	67.69 ± 1.90	66.74 ± 1.77	.85
Neutrophil	26.20 ± 1.64	27.06 ± 1.56	.91
Monocyte	5.40 ± .49	5.14 ± 0.63	.52
Eosinophil	.43 ± .13	.51 ± .12	.34
Basophil	.29 ± .09	.54 ± .14	.18

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the anterior pituitary gland (Guyton, 1986; Ganong, 1997; Norris, 1997). The number of immunopositive ACTH and GH cells decreased over time. A greater number of corticotropes and somatotropes positively stained at birth because there is a higher concentration of ACTH (Chatelain and Cheong, 1986; Apostolakis et al., 1994; Brooks et al., 1996) and GH (Adrian et al., 1983) at this time; the concentration then decreases during neonatal life. It is also possible that fewer cells were recorded because although there was hypertrophy of corticotropes and somatotropes, that field of observation remained constant and, therefore, over time fewer cells could be counted.

Adrenal gland concentrations of ACTH-R mRNA were lower in control pigs than in ACTH pigs at 1 d of age. This may be explained by the greater concentration of hypothalamic CRH and POMC mRNA, as well as the increased pituitary gland size, which suggests that the anterior pituitary gland produces and secretes more ACTH. Adrenocorticotropic hormone has been found to induce up-regulation of ACTH-R mRNA in murine and human adrenocortical cell lines (Mountjoy et al., 1994). This provides evidence that increased concentrations of ACTH up-regulated its own receptor in the adrenal gland.

The adrenal gland cortex:medulla ratio was less in control pigs than in ACTH pigs at 1 and 60 d of age. The size of the adrenal glands as not different between treatments, suggesting that internal morphologic changes occur that result in more cortical region at the expense of the adrenal medulla. The differential growth rates of the adrenal cortex and medulla zones may account for the absence of differences in COR:MED at 30 d of age between control and ACTH pigs. In control pigs CORT:MED was relatively low at birth then increased relatively quickly; conversely, the ACTH pigs were born with a greater CORT:MED that increased over a longer period. This suggests that the differences in CORT:MED between control and ACTH pigs is mainly due to prenatal development. In conditions of chronic stress, the adrenal cortex undergoes an adaptation that allows for the hypersecretion of glucocorticoids (Pignatelli et al., 1998). In ACTH pigs, maternal glucocorticoid secretion possibly affected adrenal development, resulting in a greater COR:MED. This is possible because cortisol has been shown to cross the placenta in pigs (Klemcke, 1995); therefore, it is likely that the fetal ACTH pigs were subjected to abnormal maternal glucocorticoid levels in response to the ACTH injections.

As discussed above, a number of markers of HPA function were found to be altered by restraint and exposure to ACTH. It was also found that during a stressful situation (mixing stress), control pigs had lower concentrations of plasma cortisol than ACTH pigs. This agrees with previous evidence that prenatally stressed animals are physiologically predisposed to overreact to stressful stimuli (Takahashi et al., 1990), thus resulting in a hyperactive HPA axis. This has been proven in a number of studies wherein prenatally stressed animals have increased concentrations of ACTH and(or) glucocorticoids in response to stressful situations compared with control animals (Takahashi et al., 1988; Clarke et al., 1994; McCormick et al., 1995). Because mixing of unfamiliar individuals is considered stressful to swine (Hyun et al., 1998), mixing of litters served as a stressor in this study.

There is also evidence that stress can alter multiple aspects of immune function in pigs (e.g., Kelley, 1980, 1985; Morrow-Tesch et al., 1994), whereas in other species stress has been shown to inhibit the production of pro-inflammatory cytokines important for wound repair (Kiecolt-Glaser et al., 1995) and cause decreased neutrophil function, which increases additional risks from infection after wounding (Shurin et al., 1994). To investigate the effects of the mixing stress on immune function, a small puncture was created in pigs with the use of a punch biopsy. An evaluation score of 1 was considered the best and an evaluation score of 4 was considered the worst, reflecting poor healing. Observation of punch wounds showed control pigs had an overall lower evaluation score than ACTH pigs and, specifically, control pigs had lower evaluation scores than ACTH pigs at d 9 and 11. These data indicate that punch wounds of control pigs healed at a faster rate than wounds of ACTH pigs, which may be a result of decreased pro-inflammatory cytokines. Wound healing rate differences were probably not due to decreased neutrophil function because there were no differences in the N:L.

At 1 d of age, control pigs tended to have heavier body weights than ACTH pigs. This is in agreement with previous studies that also reported that the size of prenatally stressed offspring is decreased at birth (Dahlöf et al., 1978; Pollard, 1984; Keshet and Weinstock, 1995). Because there were no differences in gestation length between control and ACTH sows, the possibility that ACTH pigs were lower in birth weight due to a shortened duration of pregnancy can be ruled out. By weaning age, the body weights of control and ACTH pigs were similar. This phenomenon has been reported in other studies in which prenatally stressed individuals with reduced birth weight "caught-up" to control offspring and both groups had similar subsequent growth rates (Pollard, 1984). The greater concentrations of IGF-I mRNA in ACTH pigs at 1 and 30 d may also explain the increase in weight gain of ACTH pigs, which normalized the body weight between treatments for the remainder of the study.

Although there were no behavioral differences at 6 wk of age between control and ACTH pigs, control pigs tended to perform a higher frequency of belly-nosing and drinking as well as a higher frequency of oral vice behaviors at 8 wk of age. Belly-nosing and oral vices are considered stereotypic behaviors because they are monotonous, abnormal behaviors or repetitive activities that seem purposeless and may have developed as a consequence of a problematic environment (Odberg, 1978; Lawrence and Terlouw, 1993; Hurnik et al.,

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1995). Although there were differences found between belly-nosing and oral vice behaviors between control and ACTH pigs at 8 wk of age, there were many other stereotypic behaviors evaluated that yielded no differences, and thus other behavioral evaluations should be employed that may better illustrate the effects on offspring whose dams were stressed during pregnancy.

Because the current results indicate that ACTH pigs had a hyperactive HPA axis, one can speculate that restraint coupled with ACTH administration to pregnant sows resulted in a surge release of glucocorticoids, of which a portion crossed the placenta to affect development and maturation of the fetal hypothalamus. It has been previously suggested that during fetal development, pulsatile release of anterior pituitary gland hormones is a result of an immature hypothalamus. These stimulatory influences are exerted from independent hypothalamic pulse generators and are modulated by the development of inhibitory feedback control in order to develop the system (Gluckman et al., 1985). In a normally developing fetus, ACTH is secreted as a result of pulsatile CRH release from the hypothalamus, and this release is regulated by negative feedback of adrenal glucocorticoids (Gluckman et al., 1985). Glucocorticoid receptors are present throughout the brain at different times throughout gestation (Rosenfeld et al., 1993). Thus, in ACTH pigs, it is possible that a surge of glucocorticoids once a week, resulting from maternal ACTH administration, would cause a disregulation of the developing fetal hypothalamus by decreasing the negative feedback effects of glucocorticoids on the hypothalamus. This occurs because once the glucocorticoid surge from the ACTH administration subsides, the now-desensitized hypothalamus would perceive low concentrations of glucocorticoids and thus increase synthesis and secretion of CRH. Weekly administration of ACTH would result in decreased negative feedback of cortisol on CRH, resulting in a continually greater release of CRH to elicit a cortisol negative feedback response. This increased CRH secretion would then overstimulate the development of the HPA axis, resulting in a hyperactive HPA axis later in life, including an increased secretion of CRH, increased pituitary gland size, increased cortex:medulla ratio, and increased cortisol release in response to stress.

In conclusion, prenatal stress in the form of restraint and weekly injections of ACTH into pregnant sows may result in a decreasing negative feedback effect of cortisol on the fetal hypothalamus, which culminates in hyperactivity of the HPA system. This hyperactivity was realized in the current study as an increase in secretion of CRH, pituitary gland size, cortex:medulla ratio, and ACTH receptor mRNA and as prolonged cortisol release in response to stress. It is possible that during stressful situations later in life this state may compromise growth, health, reproduction, or welfare. The mechanism by which and extent to which prenatal stress alters the development of the HPA axis needs to be elucidated.

Implications

Administering adrenocorticotropic hormone during restraint to pregnant sows causes effects similar to prenatal stress and results in a hyperactive hypothalamic-pituitary-adrenal axis. Consequently, when animals are stressed later in life, they may over-react to the stressor by overproduction of glucocorticoids. Because excess glucocorticoids have been shown to have a number of adverse effects, prenatal stress in swine may compromise growth, health, reproduction, or welfare. If these important production areas are affected by prenatal stress, the specific management practices that are chronically stressful to sows during gestation should be identified. If these management practices can be revised to provide a nonstressful environment for swine, animal growth, reproduction, and(or) welfare may be enhanced, and in turn the economic state of the producer may be improved.

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